COMMENTARY

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TO THE EDITOR:

Sustained prothrombotic changes in COVID-19 patients 4 months after hospital discharge

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Patients with COVID-19 are at increased risk of thrombotic complications, despite the in-hospital use of standard- or escalated-dose thromboprophylaxis.[1-3] As a result, numerous studies have focused on understanding the thrombotic tendency in these patients. Whole-blood viscoelastic tests and thrombin-generation assays have demonstrated a hypercoagulable state.[4-6] In addition, abnormalities in conventional coagulation tests, such as a prolonged prothrombin time (PT), and highly elevated von Willebrand factor (VWF), D-dimer, and fibrinogen levels have been found.[7-9] Moreover, we have recently shown that the prothrombotic changes found in COVID-19 patients shortly after admission are associated with disease severity and mortality.[10] These data align with the hypothesis that the prothrombotic changes in COVID-19 patients contribute to disease progression, potentially by facilitating pulmonary (micro)thrombosis.[11,12] Importantly, persistence of symptoms, such as dyspnea and fatigue, have been reported in a proportion of hospitalized and nonhospitalized COVID-19 patients up to 3 months after primary infection.[13-16] The pathophysiology of this so-called post–COVID-19 syndrome remains largely unknown. We hypothesize that the post–COVID-19 syndrome may involve sustained intrapulmonary activation of coagulation, perhaps driven by residual pulmonary microvascular injury, with ongoing pulmonary microthrombosis as a consequence. To the best of our knowledge, the hemostatic status of COVID-19 patients after hospital discharge has not yet been assessed. Therefore, we studied the hemostatic status of patients with a resolved COVID-19 infection. We have previously reported the hemostatic status of a cohort during hospital admission and these patients were a subset.[10] In this study, we report the hemostatic status of these patients at 4 months after hospital discharge.

We included 52 COVID-19 patients who had been admitted to the Danderyd Hospital (Stockholm, Sweden) between April and June 2020. The study complied with the Declaration of Helsinki, and informed consent was obtained from all healthy individuals and patients or, in the case of incapacity, their next of kin. The protocol was approved by the Stockholm Ethical Review Board (COMMUNITY study no. 2020-01653).

Blood samples were collected shortly after hospital admission and 4 months after hospital discharge. To assess hemostatic status, we measured various hemostatic proteins involved in platelet function, fibrin formation, and fibrinolysis; measured levels of markers for in vivo activation of coagulation and fibrinolysis; and performed ex vivo functional testing of coagulation and fibrinolysis. The specific methods and assays are described elsewhere.[10] Additionally, we measured factor V and protein C levels on an automated coagulation analyzer (STACompact 3; Stago, Breda, The Netherlands). We compared patients' hemostatic status at hospital admission with their hemostatic status at 4-month follow-up, and with 29 healthy controls using the Wilcoxon signed-rank and Mann-Whitney *U* tests in GraphPad Prism (GraphPad Software Inc, San Diego, CA), respectively.

Demographic, clinical, and routine laboratory data of patients are presented in Table 1. The healthy controls, of whom 10 (35%) were female, had a median age of 60 years (28-70 years), and had a median

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Table 1. Demographic, clinical, and routine laboratory data of study participants

Variable	COVID-19 patients, n = 52
Age, y	59 [49-63]
Female	15 (28.8)
BMI, kg/m^2	28.4 [24.7-32.4]
Comorbidity	
Cardiovascular	8 (15.4)
Diabetes	11 (21.2)
Renal dysfunction	5 (9.6)
Symptom duration, d	12 [8-15]
Duration of hospital stay, d	6 [3-13]
Days between admission and first blood sample	2 [2-3]
Location at time of first blood sample	
Ward	47 (90.4)
Intermediate care unit	4 (7.7)
Intensive care unit	1 (1.9)
Respiratory requirements at time of first blood sample	
None	20 (38.5)
Nasal cannula/mask ≤5 L O_2	23 (44.2)
Nasal cannula/mask >5 L O_2	9 (17.3)
Noninvasive ventilation	1 (1.9)
Oxygen requirement, L/min	1.3 [0.0-4.0]
Routine laboratory values at time of first blood sample	
Creatinine, mg/dL	73 [64-90]
CRP, mg/dL	98 [61-177]
Lactate, mg/dL	1.35 [1.18-1.70]
WBC count, ×10^9/L	6.5 [4.4-7.8]
Anticoagulation at time of first blood sample	
No	10 (19.2)
Standard prophylactic LMWH, 4500 IU once daily	27 (51.9)
Intermediate. prophylactic LMWH, 4500 IU twice daily	15 (28.8)
Oral anticoagulant	1 (1.9)
Anti-Xa, U/mL	0.04 [0.00-0.07]
Anticoagulation at 4-mo follow-up	4 (7.7)*
Venous thromboembolism during 4-mo follow-up	1 (1.9)

The results are presented as median [interquartile range] for continuous variables, and number (percentage) for categorical variables.

BMI, body mass index; CRP, C-reactive protein; LMWH, low-molecular-weight heparin; WBC, white blood cell.

*Two of the 4 patients already received anticoagulation prior to hospital admission. The other 2 indications for anticoagulation were venous thromboembolism and atrial fibrillation.

body mass index of 24.8 (22.5-26.6). One patient developed a deep vein thrombus with small pulmonary embolisms in the 4 months after hospital discharge. Comparisons of the various hemostatic tests of COVID-19 patients at admission and 4-month follow-up, and between patients and healthy controls, are displayed in Table 2. The PT, factor V, VWF, fibrinogen, D-dimer, and thrombin-antithrombin (TAT) levels were elevated in patients on admission, but normalized at 4-month follow-up to levels similar to those found in healthy controls. Factor VIII levels were higher at admission compared with follow-up, and remained higher than levels found in healthy controls. Levels of prothrombin and antithrombin were comparable with controls on admission, but slightly higher at 4-month follow-up. Protein C levels were lower compared with controls on admission, but higher than controls at follow-up. Despite a full normalization of TAT complex levels, ex vivo thrombin-generating potential assessed by thrombomodulin-modified calibrated automated thrombinography was markedly elevated at follow-up (Table 2). It should be noted that most of the patients (81%) received anticoagulation during hospital admission, but not at 4-month follow-up (7.7%; Table 2), suggesting a modest net decrease in the hypercoagulable state as measured by thrombin-generation assays at follow-up. Elevated thrombin-generating potential at follow-up is not associated with elevated prothrombin or decreased antithrombin or protein C levels, which are important determinants of thrombin-generating capacity.[17] Sustained elevations in factor VIII levels may, in part, explain the sustained hypercoagulable state in convalescent patients.[18] Plasminogen-activator inhibitor type 1 (PAI-1) levels were higher in patients compared with controls, both on admission and at 4-month follow-up. Plasmin-antiplasmin (PAP) complexes were highly elevated on admission but were markedly lower compared with controls at 4-month follow-up. These low PAP complex levels at follow-up align with a hypofibrinolytic state as evidenced by a prolonged clot lysis time and elevated PAI-1 levels.

In summary, COVID-19 patients have sustained prothrombotic changes as evidenced by enhanced thrombin-generating capacity and decreased plasma fibrinolytic potential at 4 months after hospital discharge. Elevated plasma levels of factor VIII and PAI-1 could in part explain the hypercoagulable and hypofibrinolytic status. Mechanisms underlying elevated factor VIII and PAI-1 levels are unclear, but may involve sustained activation of the endothelium.[19,20] Indeed, plasma levels of VWF, which is an established marker of endothelial activation, remained slightly elevated in patients at follow-up, although there was no statistically significant difference with controls. Even though these prothrombotic changes were not associated with enhanced in vivo activation of coagulation (evidenced by normal D-dimer and TAT levels), enhanced thrombin-generating capacity and a plasma hypofibrinolytic state are associated with an increased risk of thrombotic events in the general population,[21-23] and may contribute to thrombotic events in patients with COVID-19 after the resolution of their infection. Whether prolonged thromboprophylaxis following discharge is effective and safe in prevention of thrombotic events in patients with COVID-19 is currently being investigated in the ACTIV-4 trial (NCT04498273). In addition, we hypothesize that a systemic hypercoagulable and hypofibrinolytic state may result in sustained (low-grade) clot formation in the (damaged) pulmonary vasculature, which may contribute to post–COVID-19 syndrome. Whether sustained intrapulmonary microthrombosis indeed plays a role in post–COVID-19 syndrome is unclear. Additional work is needed to test this hypothesis. Nevertheless, the hypercoagulable state of COVID-19 patients at 4 months after primary infection may be clinically relevant and requires further study.

Contribution: F.A.v.M., C.T., and T.L. conceived and designed the study; S.H., A.L., and C.T. were responsible for patient inclusion; S.H. and C.T. acquired data; J.A. analyzed laboratory results;

Table 2. Hemostasis tests in COVID-19 patients on admission and at 4-mo follow-up compared with healthy controls

	Healthy controls, n = 29	COVID-19 patients on admission, n = 52	COVID-19 patients 4-mo follow-up, n = 52	Admission vs follow-up, *P*	Admission vs controls, *P*	Follow-up vs controls, *P*
Standard hemostasis tests						
PT, s	13.9 [13.5-14.6]	15.1 [14.0-16.4]	13.6 [13.2-14.3]	<.0001	<.0001	.067
Additional hemostasis tests						
Factor V, %	107 [84-124]	126 [102-141]	96 [88-107]	<.0001	.003	.079
Factor VIII, %	136 [114-157]	223 [165-286]	162 [132-201]	<.0001	<.0001	<.001
VWF, %	108 [83-128]	348 [249-427]	122 [86-154]	<.0001	<.0001	.155
Prothrombin, %	85 [82-97]	91 [81-100]	100 [91-104]	.076	.449	.001
Antithrombin, %	97 [96-104]	96 [79-106]	103 [97-111]	<.0001	.295	.030
Fibrinogen, g/L	3.14 [2.71-3.39]	6.42 [5.68-7.38]	3.27 [2.82-3.90]	<.0001	<.0001	.140
PAI-1, ng/mL	0.60 [0.10-0.75]	2.60 [1.90-4.35]	3.15 [0.85-5.58]	.857	<.0001	<.0001
Protein C, %	106 [93-119]	98 [77-115]	120 [104-129]	<.0001	.046	.051
Activation of coagulation and fibrinolysis						
D-dimer, ng/mL	290 [205-445]	1135 [660-1950]	380 [290-510]	<.0001	<.0001	.060
TAT, μg/mL	2.90 [2.05-3.80]	5.00 [4.00-7.00]	2.60 [2.33-3.38]	<.0001	<.0001	.456
PAP, ng/mL	544 [437-707]	1278 [1041-2018]	218 [157-354]	<.0001	<.0001	<.0001
Thrombin-generation assay						
ETP, nM IIa × min	606 [422-773]	811 [586-976]	851 [699-980]	.560	<.001	<.0001
Peak, nM IIa	167 [123-215]	215 [153-264]	243 [195-279]	.027	.009	<.001
Lag time, min	2.00 [1.67-2.00]	2.67 [2.28-3.00]	2.00 [1.67-2.33]	<.0001	<.0001	.108
Velocity index, nM IIa/min	77 [62-112]	100 [67-123]	138 [90-161]	<.0001	.161	<.001
Clot lysis time, min	66 [62-70]	82 [70-91]	75 [65-85]	.072	<.0001	.007

The results are presented as median [interquartile range]. Comparisons between patients on admission and follow-up were made using the Wilcoxon signed-rank test. Comparisons with healthy controls were made with the use of the Mann-Whitney *U* test. A value of $P < .05$ was considered statistically significant.

ETP, endogenous thrombin potential.

F.A.v.M. and T.L. analyzed data; F.A.v.M., S.H., J.A., A.L., M.M., N.M., C.T., and T.L. interpreted data; C.T. and T.L. supervised the study; F.A.v.M. and T.L. drafted the manuscript; and S.H., J.A., A.L., M.M., N.M., and C.T. revised the manuscript.

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